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# 붉은 빵곰팡이 細胞의 糖運搬
## —*p*H濃度와 饑餓現象이 미치는 影響—

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# Sugar Transport in Conidia of *Neurospora crassa*
## —Influence of *p*H and Starvation—

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## ABSTRACT

Uptake of $^{14}$C-sorbose and $^{14}$C-3-*O*-methylglucose by ungerminated conidia of *Neurospora crassa* was measured by means of the millipore filter technique. Initial rates of uptake of both sorbose and 3-*O*-methylglucose show a marked dependence on *p*H of the incubation medium in the range between *p*H 3.5 and *p*H 6.5. The optimal *p*H for uptake of both sugars is close to 4.75.

When ungerminated conidia are "starved" with buffer for a prolonged period of time prior to assaying their transport capacity and mycelia, no de-repression of the glucose-repressible sugar transport system is effectuated in contrast to the findings for germinated conidia.

## INTRODUCTION

In earlier publications, a system for the active uptake of sugars by cells of *Neurospora crassa* has been described (Klingmüller, 1967 a,b,c). This sugar transport system, then mainly measured by uptake of $^{14}$C-sorbose, a fructose analogue not phosphorylated in *Neurospora*, was found both in conidia pre-germinated with fructose, and also in ungerminated conidia (Klingmüller, 1968).

In germinated conidia and mycelia of the same organism an active glucose-transport system has been described by others (Scarborough, 1970 a,b; Schneider and Wiley, 1971 a,b,c). It is characterized by a high affinity to this sugar (Km=10$\mu$M) and hence was designated to the "high affinity system".

Neville *et al.* (1971) have studied on this system in ungerminated conidia. Their detailed study is of importance for the elucidation of the numbers and types of transport mechanisms involved. I, however, do not agree with those authors, in two minor aspects, the infuence of *p*H and starvation on 3-*O*-methylglucose uptake. My own data bearing on these points are communicated here.

## MATERIALS AND METHODS

Strains: *Neurospora crassa* 74-OR 23-IA

De Serres (wild type), closely related to the strain used by Neville *et al.* (1971), and sorbose resistant mutants sor$^r$-A-3/2 and B-57/3 as described in an early publication (Klingmüller, 1967 d).

Origin of conidia: They were obtained from 7 day old cultures grown either on glycerol complete agar (Horowitz, 1947; for experiments described in Figs. 1 and 2) or on Vogel's solid medium (Lester and Hechter, 1961) supplemented with 1% sucrose, which was filter sterilized (experiments described in Fig. 3). Vogel's solid medium was prepared according to the method of Neville *et al.* (1971).

Conidial suspensions were obtained in water as described before (Klingmüller, 1967 a).

*p*H measurements: *p*H-meter 25, Radiometer Copenhagen, with scale expander. Molarity of buffer ca. 0.075.

Radioactive sugars: L-sorbose-$C^{14}$ (U) and 3-*O*-methyl-D-glucose-$C^{14}$(U) were obtained from the Radiochemical Centre, Amersham, England.

Uptake measurements: The millipore filter technique was used. The radioactivity of the sample on the filter was measured either with a methane gas flow counter FH49A (Fig. 1) or in 10ml. toluene+PPO/dimethyl-POPOP with a Packard 3003 liquid scintillation spectrometer(Figs. 2 and 3).

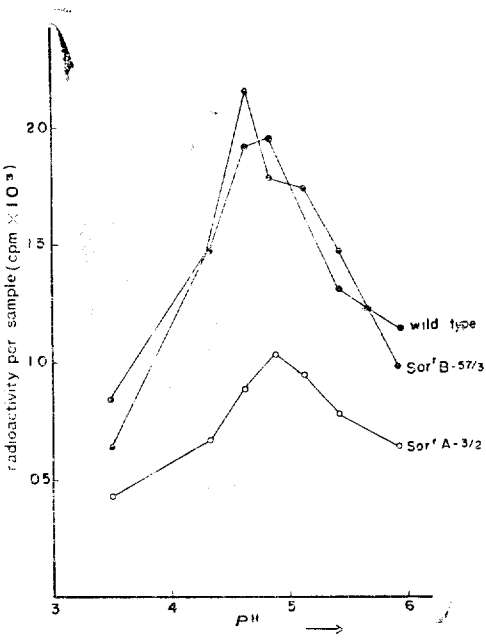


**Fig. 1.** Uptake of $^{14}$C-sorbose by conidia of three different strains at varying *p*H. Conidia from fresh suspensions were spun down and resuspended at a concentration of $4\times10^6$/m*l* in flasks with 0.075M citrate-$Na_2HPO_4$ buffer of the indicated *p*H. Here, they were equilibrated at 25± 0.1°C and constant aeration for 1 hr. The $^{14}$C-sorbose was added at a specific activity of 2.81 $\mu$C/mM to give a final concentration of $3.3\times10^{-6}$M. Incubtion was continued for 1 hr. then two samples of 5 ml each were taken from each flask, filtered onto millipore filter disks, washed twice, dried and counted. Points are averages of the two determinations.

## RESULTS

### 1. *p*H-Dependence of Transport

In earlier investigations shown in Fig. 1, there was a strong dependence of sorbose uptake on *p*H of the incubation medium with a distinct optimum close to *p*H 4.75. This was found for ungerminated wild type conidia, as well as for conidia of two different sorbose resistant mutants, as documented in the Fig. 1. In these measurements the radioactive sorbose was offered at a final concentration of ca. $3x10^{-6}$M.

Since at this concentration the rate of uptake is constant over at least two hours (Klingmüller, 1969), samples that had been incubated for 60 min. were taken to calculate that rate. Neville *et*

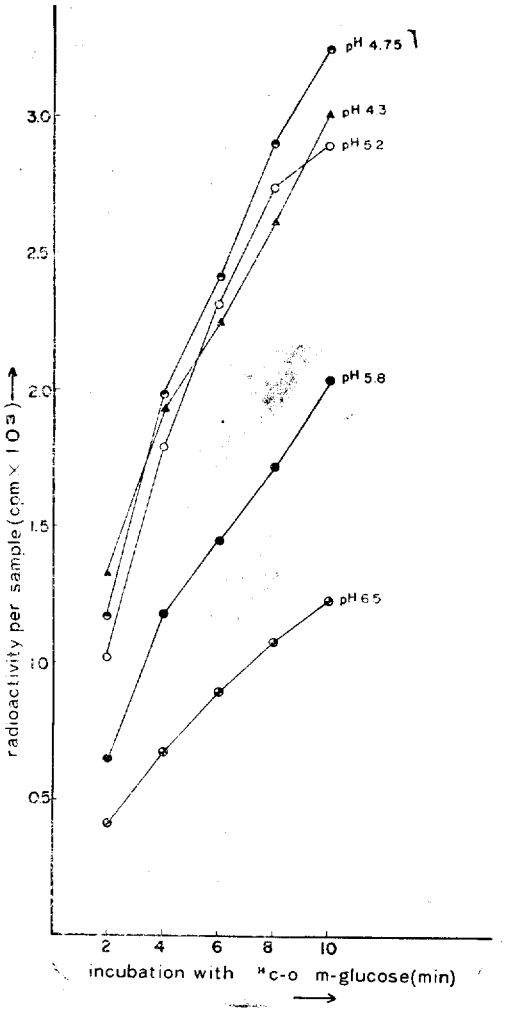


**Fig. 2.** Uptake of $^{14}$C-3-*O*-methylglucose into conidia at varying *p*H. Conidia from fresh suspensions were washed three times on millipore filters and suspended at $4\times10^6$/ml in Vogel's minimal plus 0.075 M citrate-$K_2HPO_4$ buffer of the indicated *p*H, containing in addtion $10^{-4}$M $^{14}$C-3-*O*-methylglucose at 100$\mu$C/mM specific activity. Here, they were incubated at 25±0.1°C and constant aeration. After the indicated intervals samples of 5 ml each were taken, filtered off, washed three times, dried and their radioactivity were measured. All cells were from one and the same conidial suspension.

*al.* (1971), however, used 3-*O*-methylglucose of a much higher concentration ($10^{-3}$M) and for very short incubation time up to a few minutes. This was taken into account in the experiments leading to Fig. 2. Here, too, a marked dependence

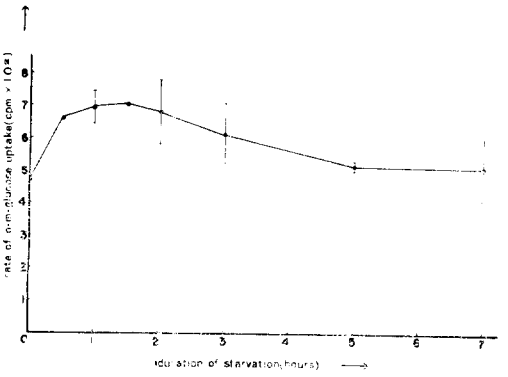


**Fig. 3.** Rate of 3-*O*-methylglucose uptake into conidia, starved different periods. Conidia from fresh suspensions were washed three times on millipore filters, and suspended for a starvation period at $8\times10^5$ m*l* in 0.015M citrate-$KH_2PO_4$ buffer of *p*H 5.5. Three experiments were carried through independently starting from different conidial suspensions.

Individual flasks were set up for each starvation time. The flasks were shaken at 25°C for the indicated periods. Then conidia were filtered off again, washed three times with buffer and transfered into the incubation flasks, containing 29.7 m*l* buffer and 0.3 m*l* $^{14}$C-3-*O*-methylglucose. Final concentration of conidia was $4\times10^6$/m*l*, final 3-*O*-methylglucose concentration $10^{-4}$ M, specific activity was 100$\mu$D/mM and incubation temperature 25±0.1°C.

For each starvation time, samples of 5 m*l* each were taken at short intervals, ranging from 0.5 to 2 min., up to 8 min. incubation. They were filtered off, washed three times with buffer, dried and counted. Rates of uptake were calculated from the activities obtained after 0.5 to 4 min. incubation, when increases of radioactivity per sample were close to linear. The points for 1 min., 1 and 2 hr. starvation and averages from 3 experiments, those for 3, 5 and 7 hr. from 2 experiments. Standard errors indicated.

on *p*H is observed with a distinct optimum close to *p*H 4.75.

**2. Effect of Starvation**

To check for an effect of starvation, the experimental procedure of Neville *et al.* (1971) was followed; e.g. *p*H was set to 5.5 and conidia were obtained from Vogel's solid medium. The temperature was kept to 25°C throughout. To compensate for the 30°C used by Neville *et al.* (1971), starvation was extended up to 7 hrs. instead of 5 hrs. It can be seen from Fig. 3 that there is an increase of uptake of 3-*O*-methylglucose during the first 30 min. of starvation. For prolonged starvation a decrease of uptake is indicated but not significant.

## DISCUSSION

Neville *et al.* (1971) are of the opinion that dependence of 3-*O*-methylglucose uptake on *p*H is very slight between *p*H 4 and *p*H 7, with an optimum of 5.0 to 5.5 for initial rate. In contrast, my data (Figs. 1 and 2) show that the uptake of both L-sorbose and 3-*O*-methylglucose into ungerminated conidia depends markedly on *p*H, and the optimum of uptake occurred close to *p*H 4.75.

This same optimum held for ungerminated conidia pre-treated with sorbose (Klingmüller, 1971), for fructose-germinated conidia with the uptake of sorbose, and for glucose-germinated conidia with sorbose and 3-*O*-methylglucose (Klingmüller and Huh, 1972). The similarity of *p*H-dependence of the uptake of both sugars in the latter case was considered as a criterion suggesting the identity of the respective transport system.

Since the results of Neville *et al.* (1971) to which I am referring here is held in general terms, there is no clear way to reconcile it with my own findings. However, their buffer was of relatively low molarity (0.01M) and conidial concentration used by them was rather high (0.5 to $2.5\times10^7$/m*l*). I have found that even with 0.075 M buffer and suspensions of no more than $4\times10^6$/m*l*, a slight drift in *p*H (0.1 to 0.2 units) towards higher value can not be avoided during 1 hr. incubation. This seems to indicate that in the experiments of Neville *et al.* (1971) *p*H was insufficiently controlled, such that the distinct *p*H optimum around *p*H 4.75 described here could not have been found them.

Neville *et al.* (1971) further stated that "conidia, incubated in citrate-phosphate buffer at 30°C for varying times (0 to 5 hrs.) before assay, exhibited an increase in the intial rate of uptake during the first 3 hrs. of prior incubation, reaching rates of 2 to 3 times of the original rate, and then decrease again". My data on the effect of starvation (Fig. 3) document a slight increase, but much earlier, and no significant decrease of the initial rate of uptake. When interpreting these discrepancies, it has to be considered that Neville *et al.*(1971), followed by me in the present study, used conidia harvested in water, washed with water, and then transferred them into buffer for starvation. Hence, the initial low uptake rates observed by both of them and me can readily be explained by the assumption that the sudden transfer of the cells from water into the starvation buffer imposed an unphysiological situation resulting in the reduction of uptake rate. The increase of this rate on prolonged incu-

bation in the buffer would then reflect a gradual recovery of cellular functions, without starvation as such being effective.

This suggesstion is supported by others (Klingmüller and Huh, 1972), in which conidia, to avoid any shock, were harvest in Fries' minimal solution (after Beadle and Tatum, 1947), washed with Fries', and starved in Fries', before transferring them into the buffered assay solution for measurement of initial rate sugar uptake. In such experiments no effect of starvation on initial rate of sorbose uptake was observed but only a slight but insignificant increase in initial rate of 3-*O*-methylglucose uptake was found. Hence the effect observed here (Fig. 3) can not be interpreted as an increase of initial rate of uptake by starvation.

I have shown, in accordance with others (Scarborough, 1970 b; and Schneider and Wiley, 1971 a,b,c), that starvation of conidia pregerminated with glucose, as well as of grown mycelia with glucose, leads to a strong increase of initial rate of 3-*O*-methylglucose uptake. This effect, as demonstrated by Schneider and Wiley (1971 c), is due to the de-repression of the "high affinity system" mentioned above. From the present data and other data (Klingmüller and Huh, 1972) it follows that ungerminated conidia as opposed to germinated ones are not amenable to this de-repression. It may be speculated that "high affinity system" might be effective. This secondary regulatory mechanism should prevent the de-repression of the "high affinity system" as long as the conidia do not germinate.

## 摘　　要

發芽되지 않은 붉은빵곰팡이(*Neurospora crassa*)의 conidia가 $^{14}$C-sorbose나 $^{14}$C-3-*O*-methylglucose를 흡수하는 것을 millipore filter technique로 측정하였다.

Sorbose나 methylglucose가 細胞內에 운송되는 최적 *p*H는 4.75이다.

發芽되지 않은 conidia를 buffer용액에서 기아를 시켰을 때 發芽된 conidia나 mycelia에서 관찰된 것과는 달리 그들의 糖運送能力은 de-repression이 되지않고 제 2의 조절기작으로서 運搬되고 있다.

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